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Anti-inflammatory Activity of Ethanolic and Aqueous extracts of *Toxocarpus beddomei Gamble*

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A B S T R A C T

In this study anti-inflammatory activity of ethanolic and aqueous extracts of *Toxocarpus beddomei Gamble* belonging to family apocynaceae were studied in. Preliminary phytochemical screening revealed the presence of various vital components. The ethanol & aqueous extracts of *Toxocarpus beddomei Gamble* was found to be effective in a dose dependent manner against Carrageenan induced rat paw oedema and cotton pellet induced granuloma on experimental rats at the dose of 200 mg/kg and 400 mg/kg body weight, The extract produced a significant decrease in the inflammation in terms of reduction in the paw oedema and granuloma in albino rats. At the same dose, the extract showed significant anti-inflammatory activity comparable to that of the standard drug Indomethacin.

Keywords: *Toxocarpus beddomei Gamble*, Anti-inflammatory, Apocynaceae, Paw oedema, granuloma

A R T I C L E I N F O

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1. Introduction

Inflammation is normal and necessary protective response to the harmful stimuli such as infectious agents, antigen-antibody reactions, thermal, chemical, physical agents, and ischemia [1]. It is caused by a variety of stimuli, including physical damage, UV irradiation, microbial attack, and immune reactions. The classical key features of inflammation are redness, warmth, swelling, and pain. Inflammation cascades can lead to the development of diseases such as chronic asthma, arthritis, multiple sclerosis, inflammatory bowel disease, and psoriasis. Many

of these diseases are debilitating and are becoming increasingly common in our ageing society. Rheumatoid arthritis and degenerative arthritis are the major inflammatory diseases affecting people worldwide [2].

Rheumatoid arthritis is an inflammatory term that usually involves multiple joints. It affects 0.3–1.0% of the worldwide population and is more predominant among women in developed nations.The continual inflammation leads to joint damage, however the disease can be inhibited with drugs uses. Degenerative joint disease, which is considered by trouncing of joint cartilage that leads to pain loss and damage the function primarily in the hips and, affects 9.6% of adult males and 18% of women aged more than 60 years. Gains in life expectancy and aging populations are required to make the fourth leading cause of handicap by the year 2020 [3].

Pathophysiology:

All inflammatory diseases have almost a common pathway of generation of disease, which involves generation of various inflammatory mediators at various stages due to initial stimulation by one or various etiological factors which may be an infection, an injury or even an allergic stimulus. The etiological agent causes increased vascular permeability after initial vasodilation and increased blood flow in the area due to release of various substances including Histamine from the mast cells in the areas. The increase in vascular permeability may be due to formation of endothelial gaps under the influence of Histamine, Leucotrienes, Bradykinins or Substance P or it may also be because of transcytosis which is due to intacellular formation of vesiculovacuolar organelles across the endothelial cells under the influence of VEGF and other factors. These vesiculovacuolar organelles act as channels across the endothelial cells increasing vascular permeability. It may also be because of endothelial retraction or cytoskeletal reorganization which creates gaps in between the endothelial cells under the influence of TNF, IL-1, IFN-U. It may also be because of leucocytes mediated lysosomal and proteolytic injury [4]. Due to increased permeability and increased vascular blood flow, protein rich plasma starts exuding into the intracellular spaces. Simultaneously, accumulation of RBCs in the centre of blood vessels and marginalization of leucocytes to the periphery starts, where the leucocytes then show adhesion to the capillary walls with the help of receptors on the endothelial cells to which these cells bind through various adhesion molecules. The major adhesion receptors and adhesion molecules are selectins (P-selectin and E-selectin receptors on the endothelial cells and LAM adhesion molecule on the leucocytes), Immunoglobulins (ICAM-1 and VCAM- 1 receptors on the endothelial cells), integrins (MAC-1 and VLA-4 on leucocytes binding to I-CAM and V-CAM respectively on the endothelial cells) and other adhesion molecules which include mucin like glycoproteins [5].

The adhesion process is stimulated by Histamine and PAF which induce reorientation of P-Selectins, cytokines (IL-1 etc) in which increase synthesis of adhesion molecules (E-Selectin, ICAM, VCAM) and then adhesion by various adhesion molecules and receptors.

The WBC then show transmigration or movement outside through the capillary walls into the interstitial spaces, the process being helped by various mediators particularly CD31, and PECAM [6]. The process is by pseudopod formation by the cells [7]. After extravassation from the blood vessels into the tissue fluid, the leucocytes are further attracted to the site of inflammation by various chemotactic agents, which include endogenous substances, e.g. chemokines as well as bacterial products acting as chemotactic substances [8]. The various endogenous substances include members of the complement system e.g., C_5A, products of lipoxygenase pathway, e.g., LTB4 and cytokines e.g. IL_8. The binding with chemokines causes leucocytes to release more intracellular calcium by conversion of Phosphoinolpyruvate to Inositol pyrophosphate under the influence of phospholipase enzyme. This increased intracellular calcium stimulates contraction and pseudopod formation and movement towards the inflammatory area.

Subsequently, by the influence of chemokines, arachidonic acid metabolites are released by the leucocytes by the hydrolytic action of phospholipases on phospholipids and then converted to various eicosanoic acid derivatives. These include prostacyclins, prostaglandins and thromboxanes and finaly various leucotriene derivatives. Side by side, there is release of lysosomal enzymes from the inflammatory cells by degranulation of lysosomal granules present within these cells. The leucocytes then start a process of phagocytosis of exogenous inflammatory agents after opsonisation of these substances with opsonins e.g., C_3b, Fc, and Collectins [9]. After opsonisation, engulfment of the inflammatory agents and simultaneous release of lysosomal hydrolytic enzymes takes place. These hydrolytic enzymes or hydrolases along with various oxidases cause hydrolytic and oxidative degradation of the causative agents into smaller components which are later desposed off. Simultaneously, the inflammatory injury may start resolving itself by new tissue formation and angiogenesis (if required), or it may start showing chronic inflammatory patterns and appearance of fibrotic scars characteristic of the type of tissues and the eteological causes. Various characteristic tissue changes may be observed particularly in synovial structures related to type of inflammation and eteological causes and are characterized as various forms of arthritis [10]. The formation of eicosanoids is critical to the progress of various stages of inflammation as these mediators affect every step of inflammation.

2. Materials and Methods

Collection of Plant Material: The *Toxocarpus beddomei Gamble* was collected from the tribal belts of the local area of Kanniyakumari district, Tamilnadu, India. The plant was identified, confirmed and authenticated by Dr. Madhava

Chetty, Assistant Professor, Department of Botany, Sri Venkateswara University, Andhra Pradhesh. After authentification the whole plant of *Toxocarpus beddomei Gamble* were collected in bulk and washed under running tap water to remove adhering dirt. Then leaves were shade dried. The dried materials were made into coarse powder by grinding in mechanical grinder and stored in a closed air tight container for further use.

Preparation of Extracts

The coarse powder was taken in Soxhlet apparatus and extracted successively with ethanol, ethyl acetate, n-butanol and petroleum ether as solvent. A total amount of 650 g coarse powder was extracted with 1200 ml of each solvent. For each solvent,10 cycles were run to obtain thick slurry. Each slurry was then concentrated under reduced pressure to obtain crude extract. All crude extracts were kept in closed air tight containers under cool and dark place for further study.

Grouping of animals: Each group was allotted six animals each. Group I: Received 3% aqueous suspension of gum acacia (1ml/200g) as vehicle, Group II: Received standard drugs, Group III: Received EETB (200 mg/kg), Group IV: Received EETB (400 mg/kg), Group V: Received AETB (200 mg/kg) and Group VI: Received AETB (400 mg/kg).

Carrageenan induced rat paw oedema

The initial right hind paw volume of the rats were measured using a plethysmometer and then 0.1 ml of 1% (w/v) carrageenan was subcutaneously injected into the subplantar region of the right hind paw. The volume of right hind paw was measured at 1, 2, 3, and 4 h after carrageenan injection, and the edema volume was determined. The data were expressed as paw volume (ml), compared with the initial hind paw volume of each rat. Co solvent, alcoholic extracts (200 & 400 mg/kg) of *Toxocarpus beddomei Gamble* and aqueous extracts (200 & 400 mg/kg) of *Toxocarpus beddomei Gamble*, as suspension in distilled water and indomethacin (10 mg/kg) was orally administered 30 min before carrageenan injection. Each group comprised of 6 rats. The group received co solvent was treated as control [11-14].

Cotton pellet granuloma

Cotton pellet granuloma was induced according to the method of D' Arcy et al. Sterilised cotton pellets each weighing 10mg were implanted in both axilla and groin of each rat under light ether anaesthesia. Twenty four rats were divided into four groups as shown in for various treatments for five days. Subsequently, on 6th day all pellets were dissected out under ether anaesthesia and dried at 70^{o}C for 6 hours and weight of each granuloma was determined [15-17] (Table 2).

3. Results and Discussions

Statistical analysis

The data represent mean ± SEM. The results were analyzed statistically using one-way ANOVA followed by Dunnett's test. The minimum level of significance was set at $p < 0.05$. All assays were conducted in triplicate and statistical analysis was done, using Graph pad Prism (version 5) software.

Table 1: Effect of *Toxocarpus beddomei Gamble* extracts on Carrageenan induced rat paw oedema in albino rats

Treatment	Dose mg/kg	Percentage of inflammation at time (h)			
		1	2	3	4
Control	5ml/kg	37.51 ±4.65	85.66±3.11	106.65±6.14	126.81±6.10
Indomethacin	10	18.15±3.32*	21.08±3.60***	25.67±3.46***	31.50±3.45***
EETB	200	32.65±5.50	63.28±4.08**	90.48±3.56	101.87±5.92**
EETB	400	17.35±3.45**	52.50±4.74***	82.42±5.18**	90.27±4.94***
AETB	200	30.48±2.78*	60.12±3.58*	92.22±3.95**	103.22±3.59**
AETB	400	16.65±2.86**	43.19±2.78**	83.46±2.46*	91.11±4.15***

Each value represents mean±SEM of 6 observations. *p<0.05, **p<0.01, ***p<0.001 vs control, n=6
Data was analyzed using One-way ANOVA followed by Dunnett's test.

Table 2: Effect of *Toxocarpus beddomei Gamble* extracts on cotton pellet induced granuloma in albino rats

Treatment	Weight of granuloma (mg)	Pair wise mean difference
Control	34.43 ± 2.39	
EETB (200mg/kg)	23.36*** ± 0.58	10.16 ± 2.27
EETB (400mg/kg)	20.76*** ± 1.08	11.66 ± 2.27
AETB (200mg/kg)	24.41**±1.12	09.97±2.16
AETB (400mg/kg)	21.22***±2.13	10.19±2.19
Indomethacin (5mg/kg)	18.26*** ± 1.62	14.26 ± 2.27

Each value represents mean±SEM of 6 observations. *p<0.05, **p<0.01, *** p<0.001 vs control
Data was analyzed using One-way ANOVA followed by Dunnett's test.

4. Conclusion

The preliminary phytochemical screening of *Toxocarpus beddomei Gamble* extracts showed the presence of alkaloids, tannins, Carbohydrates, flavonoids, terpenoids and saponins. These constituents may be responsible for the *in vivo* anti-inflammatory activity of *Toxocarpus beddomei Gamble*. Among all the extracts ethanol & aqueous

extracts showed dose dependant & significant anti-inflammatory activity as compared to reference drug loperamide. The folklore claim of *Toxocarpus beddomei Gamble* used as an anti-inflammatory have been confirmed.

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